How and What to Brew with *S. eubayanus*

Homebrew Con 2016

Jared Spidel

Saccharomyces spp.

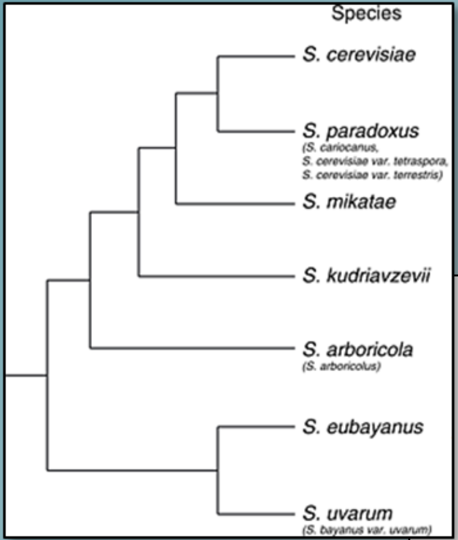
Many species described, but turned out to be divergent strains or hybrids of previously described species

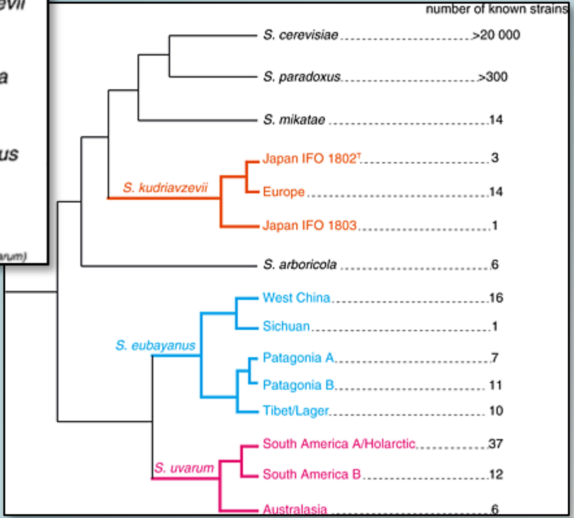
Interesting early 20th century read: “The Yeasts”, Alexander Guilliermond, 1920 https://archive.org/details/cu31924000078810

Seven known species of *Saccharomyces*

S. cerevisiae and *S. uvarum* widely associated with fermentations

S. paradoxus, *S. mikatae*, *S. kudriavzevii*, *S. arboricola*, *S. eubayanus* never found in association fermented beverages





Boynton & Greig. Yeast. Dec;31(12):449-62. 2014.
Barnett. Yeast; 21: 1141–1193.2004.
Kurtzmann, Fell, Boekhout (eds.) The Yeasts, 5th edn,Vol. 2. 2011

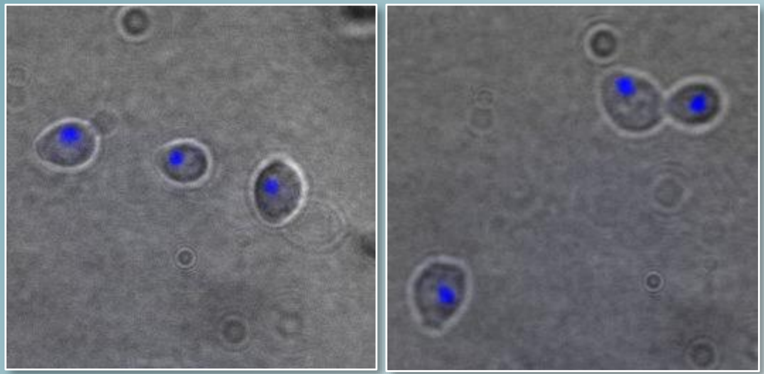
Discovery of *S. eubayanus*

Discovered by yeast hunters in search of new *Saccharomyces* species

Found associated with southern beech trees in Patagonia[1], oak and deciduous trees in Tibet and western China[2], North American beech trees in Wisconsin[3], and the forests of North Island New Zealand[4]

Genetic analysis demonstrated *S. eubayanus* is long lost parent of lager yeast *S. pastorianus*

S. eubayanus *S. cerevisiae*



DAPI, Brightfield 100x

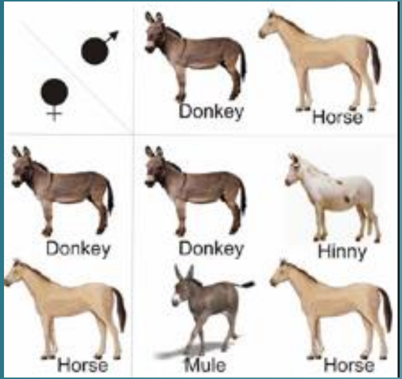
1. Libkind et al. Proc Natl Acad Sci U S A 108(35), 14539-14544. 2011.
2. Bing et al. Curr.Biol 24(10), R380-R381. 2014.
3. Peris et al. Mol Ecol. 23(8), 2031-2045. 2014.
4. Gayevskiy & Goddard. Environ. Microbiol. 2016.

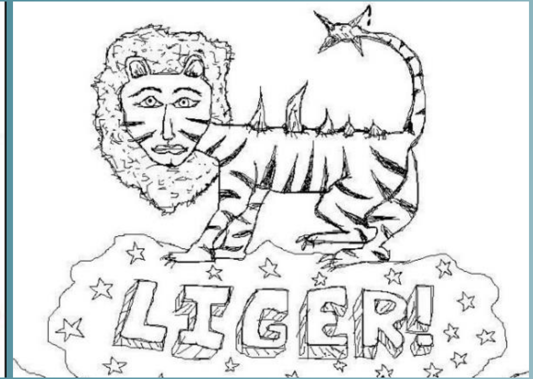
Interspecies Hybridization

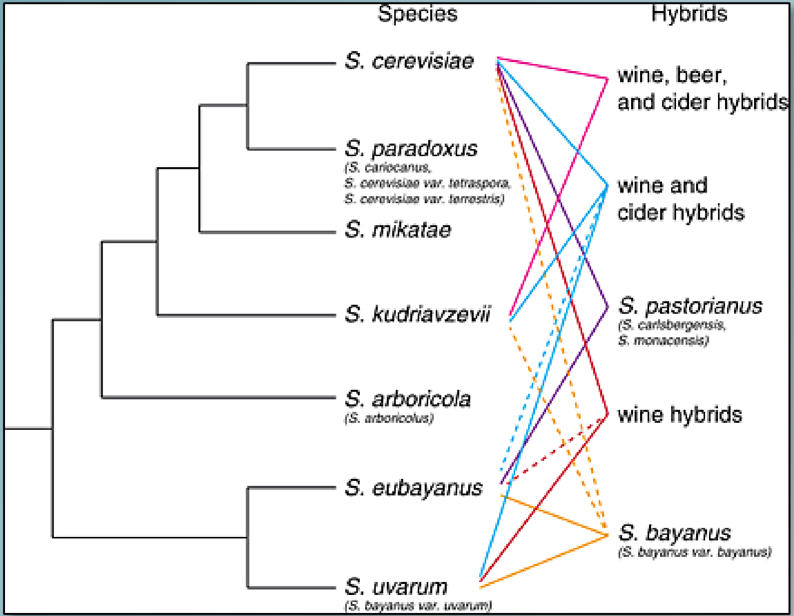
Domestication sometimes resulted in hybridization between two species.

Mule, wheat, peppermint, grapefruit

Usually sterile and require human cultivation or husbandry







Yeast Hybridization

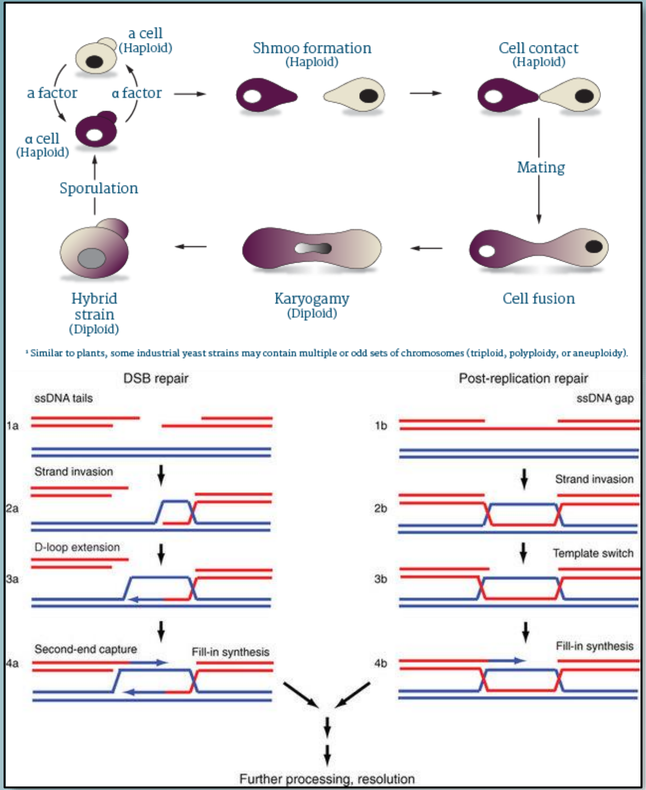
Prior to late 19th century all fermentations were a mixture of yeast strains and species

Under stress, *Saccharomyces* reproduces sexually

Results in diploid (2 sets of chromosomes)

Can also result in polyploidy hybrids

Over time, resulting from selective pressure, duplicate chromosomes can chimerize via homologous recombination or can be simply deleted.



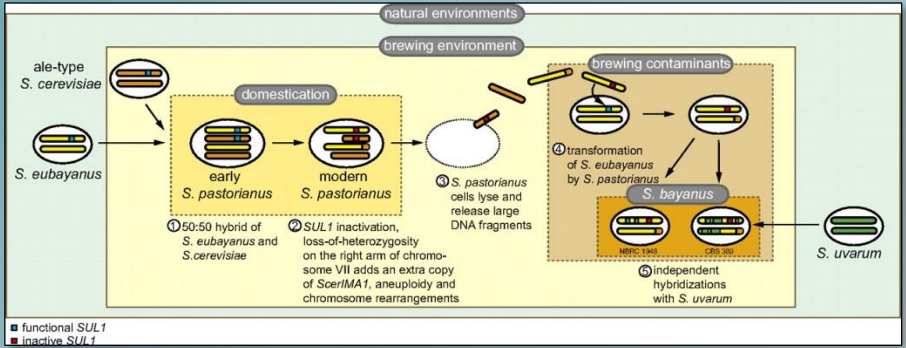
Yeast Domestication

Since the early 20th century suspected that *S. pastorianus* was not true species – low sporulation, low viability

Mid-1980s genetic analysis demonstrated as a hybrid of *S. cerevisiae* and likely the brewing contaminant *S. bayanus*

In 2011 non-*S. cerevisiae* identified as *S. eubayanus*

S. bayanus is a hybrid of *S. cerevisiae, S. uvarum*, and *S. eubayanus*



A model of the formation of S. pastorianus and the hybrid strains of S. bayanus. First, wild S. eubayanus and ale-type S. cerevisiae hybridized to form an allotetraploid that gave rise to S. pastorianus. Second, domestication imposed strong selective pressure for strains with the most desirable brewing properties. Third, in the brewing vats with high densities of S. pastorianus, cell lysis releases large DNA fragments that occasionally transform, fourth, contaminating wild strains of S. eubayanus because of the lack of pure culture techniques. Fifth, multiple hybridization events with wild strains of S. uvarum gave rise to CBS 380T and NBRC 1948. This model does not exclude prior or parallel involvement of S. uvarum in brewing or contamination.[1]

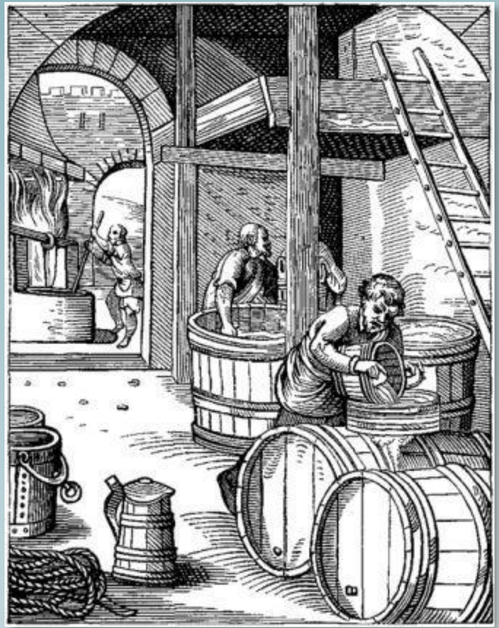
1. Libkind et al. Proc Natl Acad Sci U S A 108(35), 14539-14544. 2011.

A Brief History of Lager Brewing

In 15th-century Bavaria, brewers began fermenting and storing beer at colder temperatures, and in 1553 summer brewing was banned

The process of selecting cryotolerant yeast resulted in new strains

The mixture of microbes in primitive "yeast" contained other *Saccharomyces* species that mated with *S. cerevisiae* to create hybrid strains, and under certain conditions these hybrid strains out-competed wild-type *S. cerevisiae*.



Duke Albrecht V of Bavaria confined brewing between St. Michael's Day (Sept 29) and St. George's Day (April 23)

The Silk Road

After discovery in Patagonia, theories about how *S. eubayanus* traveled to Bavaria

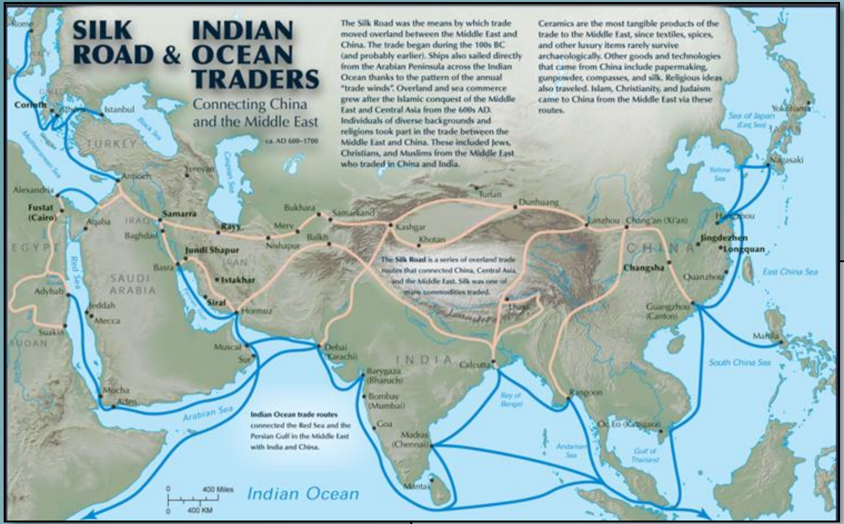
Never found in Europe… yet

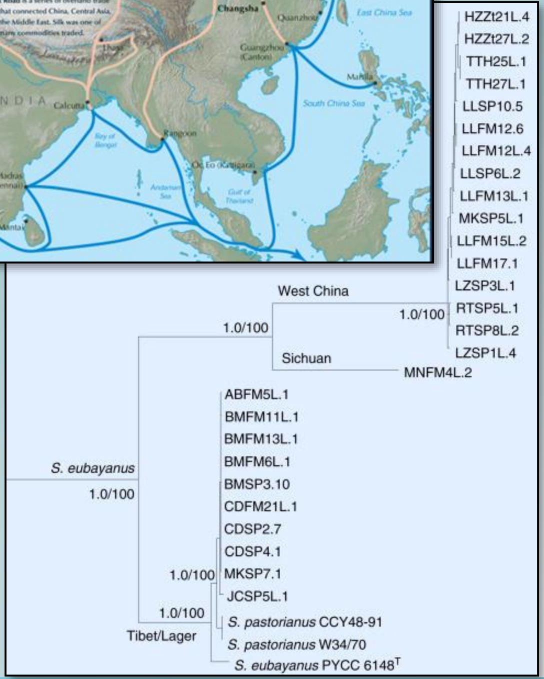
Identification of Chinese/Tibetan strains closer related to *S. pastorianus* (99.82% vs 99.35% identity to Weihenstephan 34/70)[1]

Travelled to Europe via Silk Road?

Many of the Chinese/Tibetan strains poorly utilize maltose[2]

Its only chance at survival was hybridizing with *S. cerevisiae*



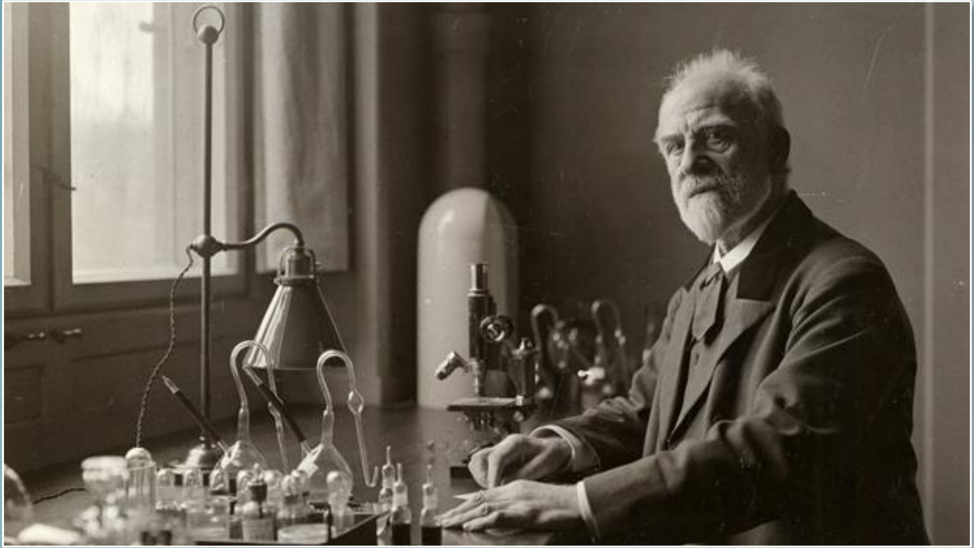


1. Bing et al. Curr.Biol. 24(10), R380-R381. 2014.
2. Personal communication, K. Krogerus

Isolating Pure Yeast Cultures

Emil Christian Hansen isolated the first lager strain (*S. carlsbergensis*; CBS1513), and freely distributed it to other breweries.

"On November 12, 1883 the Old Carlsberg Brewery started to use in its production Unterhefe Nr. 1. In 1884 the entire production of 200,000 hl beer was based on pure strains of yeast, as was the almost equal quantity manufactured at the New Carlsberg Brewery of Carl Jacobsen. Within a few years the use of clones of bottom fermenting yeast in beer production became the standard procedure throughout the world. By 1892 Pabst, Schlitz and Anheuser-Busch in North America alone manufactured 2.3 million hl with pure yeast strains as did an additional 50 breweries on that continent."[1]



1. von Wettstein D. Proceedings of the 21st European Brewery Convention Congress; pp. 97–119. 1983.

Isolating Pure Yeast Cultures

At the turn of the 20th century, Paul Lindner isolated two individual *S. pastorianus* strains.

Named Saaz (type 1) and Frohberg (type 2) after their respective regions.

Bohemian and Carlsberg breweries adopted Saaz strains, while most other breweries in Denmark, the Netherlands and Germany adopted the Frohberg strains.

Lindner P. 1909. Mikroskopische Betriebskontrolle in den Garungswerben, 6th edn. 1909.

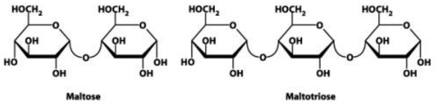
Saaz and Frohberg Strains

Saaz strains are more cryo-tolerant and flocculent than Frohberg strains, although less attenuative due to inability to ferment maltotriose.

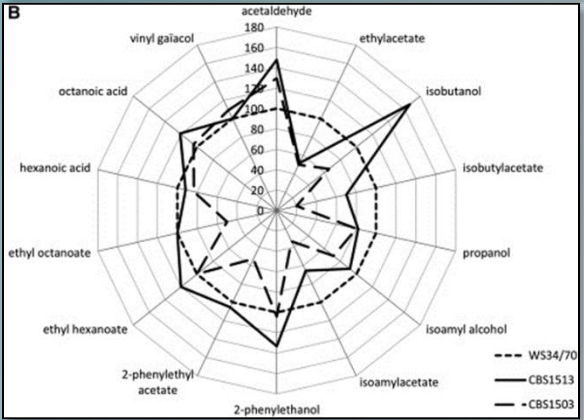
“[Saaz] lager yeasts showed greater amounts of acetaldehyde (perceived as fruity at these concentrations) whereas the [Frohberg] strain produced far more ethylacetate (pear drops flavor) and also more isoamyl alcohol/acetate (banana flavor).”[1]

	Strain	Maltose (g/L)	Maltotriose (g/L)
none	15 °P wort	68.5	18.9
Ale	A56 22 °C	0.2	14.2
	A60 10 °C	58	18
	A60 22 °C	0.3	6.3
Frohberg	A03 10 °C	0.4	8.6
	A03 22 °C	0.3	5.2
	A15 10 °C	0.2	6.6
	A15 22 °C	0.3	3.1
Saaz	A11 10 °C	4	18.7
	A11 22 °C	0.4	18.7
	A12 10 °C	18.1	18.7
	A12 22 °C	13.4	18.6

	Strain	Maltose uptake µmol/min/g	Maltotriose uptake µmol/min/g
Ale	A60	28	8.2
Frohberg	A15	28	6.4
Saaz	A11	47	0.5
	A12	37	0.3
	A53	40	0.4



22 °C = 71.6 °F
10 °C = 50 °F



WS34/70 – Frohberg
CBS1513 and CBS1503 – Saaz

1. Walther et al. G3 (Bethesda). Feb 27;4(5):783-93 . 2014

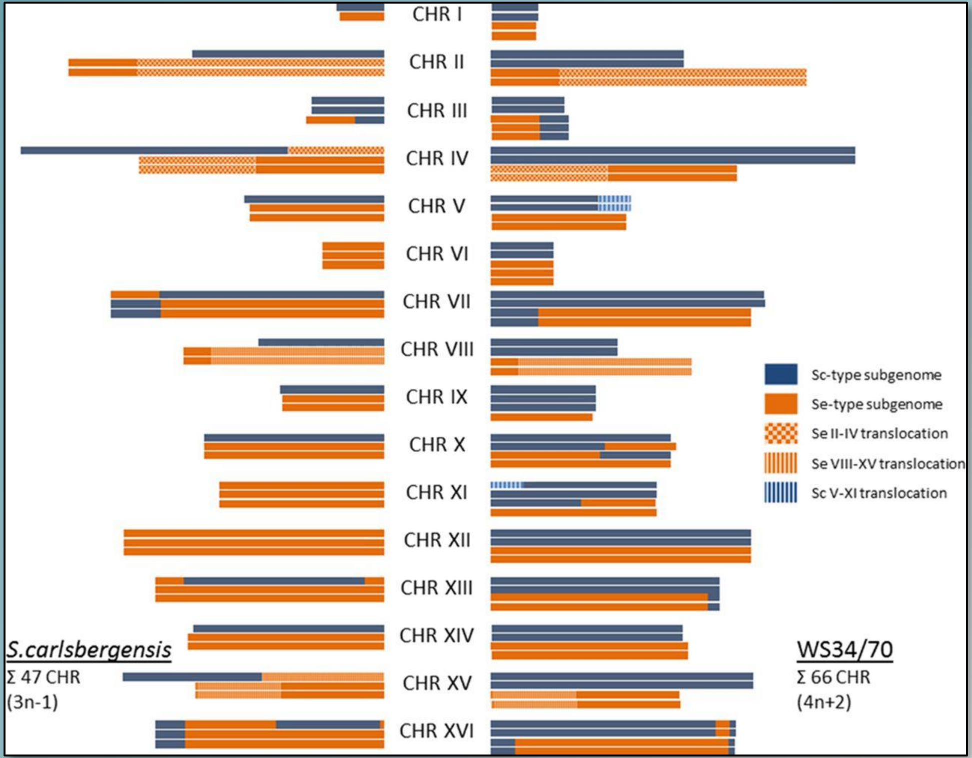
Some Quick Genetics

Saaz stain CBS1513 contains 47 total chromosomes (triploid), mostly from *S. eubayanus*[1]

Frohberg strain WS34/70 is tetraploid – basically two diploid *S. cerevisiae* and *S. eubayanus* genomes[2]

Some variability in chromosome number within Frohberg strains[3]

Mitochondria of both strains derived from *S. eubayanus*[2,4]

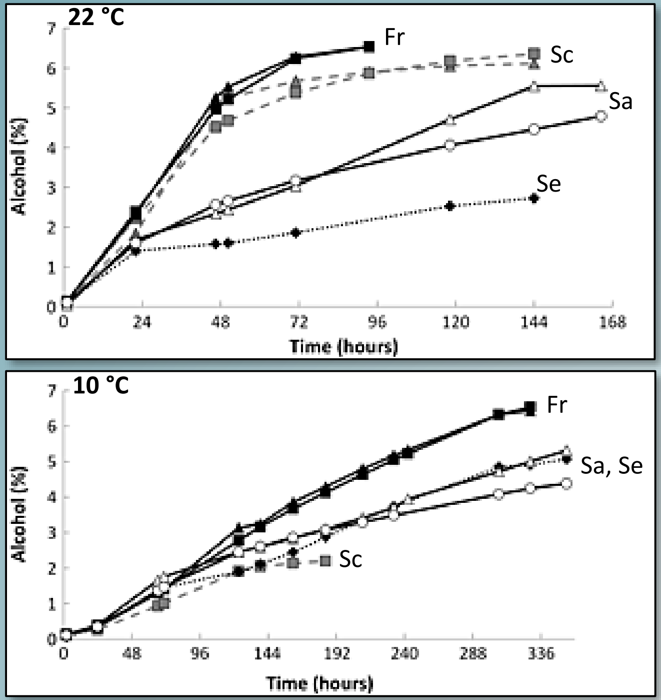


Blue – *S. cerevisiae* Orange – *S. eubayanus*[5]

1. Walther et al. G3 (Bethesda). Feb 27;4(5):783-93 . 2014
2. Nakao et al. DNA Res. Apr;16(2):115-29. 2009
3. van den Broek et al. Appl Environ Microbiol. Sep;81(18):6253-67. 2015
4. Baker et al. Mol Biol Evol. Nov;32(11):2818-31. 2015
5. Wendland. Eukaryot Cell. Oct;13(10):1256-65. 2014

Fermentation by *S. eubayanus*

- Comparison of Saaz and Frohberg strains with *S. eubayanus*
- Little fermentation by *S. eubayanus* at warm temperatures
- Saaz and *S. eubayanus* behave very similarly
- Frohberg had high attenuation, but took longer to begin fermentation at colder temperatures



Fermentation (alcohol % by volume) of 15 °P all-malt wort at 22 °C or 10 °C. Strains are the *S. cerevisiae* ale strains (Sc), the *S. eubayanus* type strain (Se), the *S. pastorianus* Frohberg-type lager yeast (Fr) and the *S. pastorianus* Saaz-type lager yeast (Sa)

Gibson et al . Yeast. Jul;30(7):255-66. 2013.

Fermentation by *S. eubayanus*

No fermentation of maltotriose by Saaz strains or *S. eubayanus*

Saaz strains produced esters ethyl acetate (fruit, solvent), 3-methylbutyl acetate (banana, pear) and ethyl caprylate (apple, aniseed) below taste threshold levels in finished beers (30 mg/L, 1.2 mg/L, and 1 mg/L, respectively)

Frohberg and *S. eubayanus* fermentation flavor profiles very similar, but Saaz and *S. eubayanus* fermentation kinetics very similar

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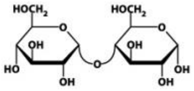
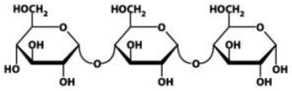
	A11 22 °C	0.4	18.7
	A12 10 °C	18.1	18.7
	A12 22 °C	13.4	18.6
S. eubayanus	C902 10 °C	6.8	18.8
	C902 22 °C	46.9	18.5

	Strain	Maltose uptake µmol/min/g	Maltotriose uptake µmol/min/g
Ale	A60	28	8.2
Frohberg	A15	28	6.4
Saaz	A11	47	0.5
	A12	37	0.3
	A53	40	0.4
S. eubayanus	C902	38	0.4



Maltose



Maltotriose

	Ale		Frohberg				*S. eubayanus*	Saaz			
	A56	A60	A03		A15		C902	A11		A12	
	22 °C	22 °C	10 °C	22 °C	10 °C	22 °C	10 °C	10 °C	22 °C	10 °C	22 °C
Ethanol (original)	6.1	6.4	6.4	6.5	6.5	6.5	5.1	5.3	5.6	4.4	4.8
Acetaldehyde	14	99.9	32.2	15.5	32	12.6	52	58.3	22.3	8.6	49.4
1-Propanol	13.5	17.4	6.3	13.1	6.8	15.2	7.4	6.1	16.2	6.1	11.4
2-Methylpropanol (isobutanol)	14.4	16.7	11.2	14.5	11.4	16	13.9	5.8	46.2	10.7	13.7
3-Methylbutanol (isoamyl alcohol)	24	13.6	15.9	22.6	19.6	29.4	18.7	12.2	30.7	10.4	16.2
2-Methylbutanol	26.4	18.3	25.6	28.7	22.8	34.3	29.5	11.4	53.1	11.5	21.2
2-Phenylethyl alcohol	7.8	2.6	1	1.4	1	1.2	1.2	1.1	1.6	1.6	2
Ethyl acetate	25.2	20.8	17.6	26.6	22.8	24	23.4	9.5	16.2	4.3	13.2
3-Methylbutyl acetate (isoamyl acetate)	1.8	0.7	1	2.2	2.2	2.1	1.7	0.3	0.4	0.3	0.3
Ethyl caproate (ethyl hexanoate)	0.1	0.4	0.3	0.2	0.3	0.2	0.5	0.5	0.1	0.2	0.4
Ethyl caprylate (ethyl octanoate)	0.4	0.2	0.6	0.4	0.4	0.4	0.7	0.1	0.1	0.2	0.1
2-Phenylethyl acetate	0.5	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1
Ethyldecanoate	3.4	0.8	1	0.6	0.9	0.8	0.9	0.4	0.5	0.4	0.8

Gibson et al . Yeast. Jul;30(7):255-66. 2013.

How to Brew with *S. eubayanus*

Culturing *S. eubayanus*

S. eubayanus (CBS 12357/PYCC 6148)

Grows well on YPD-agar plates, in YPD medium, and 1.040 wort

Grows similarly at 4 °C (39 °F) & 22 °C (72 °F)

In my hands, density tends to be higher than *S. cerevisiae*

In 1.040 wort and grown on stir plate at 18-22 °C, *S. cerevisiae* averages 100-150x10^6 cells/ml, *S. eubayanus* averages ~300x10^6 cells/ml

S. eubayanus Flavor Profile

German Pilsner

High IBU to balance low attenuation

Clean malt and hop profile to really taste the yeast’s flavor profile

Apple/pear esters

Some sharp phenols

Unrefined, muddy flavor

Match these flavors to the best recipe

Eubayanus Pils

100 % Pilsner Malt
- 66 °C (151 °F), 1 hour

Hops: 40 IBU, 0.78 BU:GU
Sterling: 13.6 IBU @ 60 min, 16.4 IBU @ 20 min, 9.8 IBU @ 10 min

Fermented 10 °C (50 °F), 3 weeks, racked and lagered

SG: 1.051
FG: 1.020
61% attenuation
4.1% ABV

Collecting Brewing Data

Little information on practical brewing with *S. eubayanus*

Info from lab data using 15 °P (1.061) wort (unhopped?) fermented at 10 °C (50 °F) 1-2 weeks

Analysis on unconditioned beer

Optimal mash temp/time, fermentation temp/time, IBUs, types of malt and hops, etc?

Rather than test one variable at a time (hundreds or thousands of beers), setup brewing competition

The Patagonian Brewing Experience

BUZZ, Stoney Creek, Keystone Hops clubs

Brew anything – except maltose is only fermentable and only yeast is *S. eubayanus*

Transferred yeast to White Labs

Design of Experiments

Test of Means - one factor experiment

Multi-Factor Experiments

- Full Factorial experiment – 2^k, 3^k, 4^k, etc; k = # factors
- 10 factors at 2 levels requires 1024 runs
- DOE - fractional factorial designs may be used

Randomization of factor levels between runs

Factors:

Original Gravity
Starter – Yes or No
Starter Type
All Grain/Extract
Malt Bill
Mash Time
Mash Temp
IBUs
Fermentation Time
Fermentation Temp

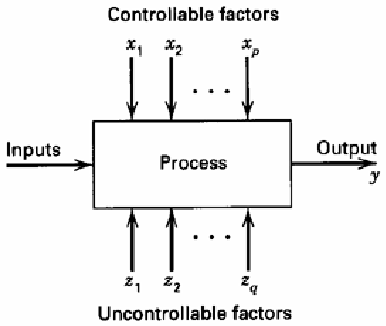


Figure 1-1 General model of a process or system.

Data Analysis

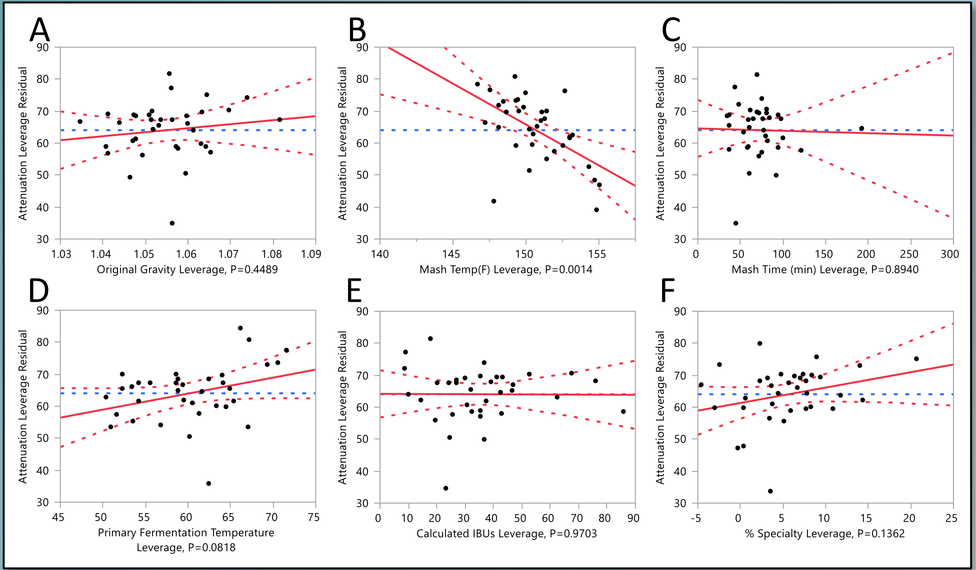
Analyzed attenuation output as a function of OG, mash temp, mash time, fermentation temp, IBUs, percentage of specialty malts

Attenuation – mean 63.57%, median 64.86%, standard deviation 11.66%, CI 3.30%

Correlation between increased attenuation and mash temp

No significant correlation between fermentation temp and attenuation

Contrary to published reports good attenuation up to 22 °C (72 °F)



CO_2 May Affect Attenuation

Closed versus open fermentation

Closed – lid with airlock

Open – lid set on top of bucket

After 2 weeks, 10-point difference

Changed "closed" to "open" and next day fermentation restarted

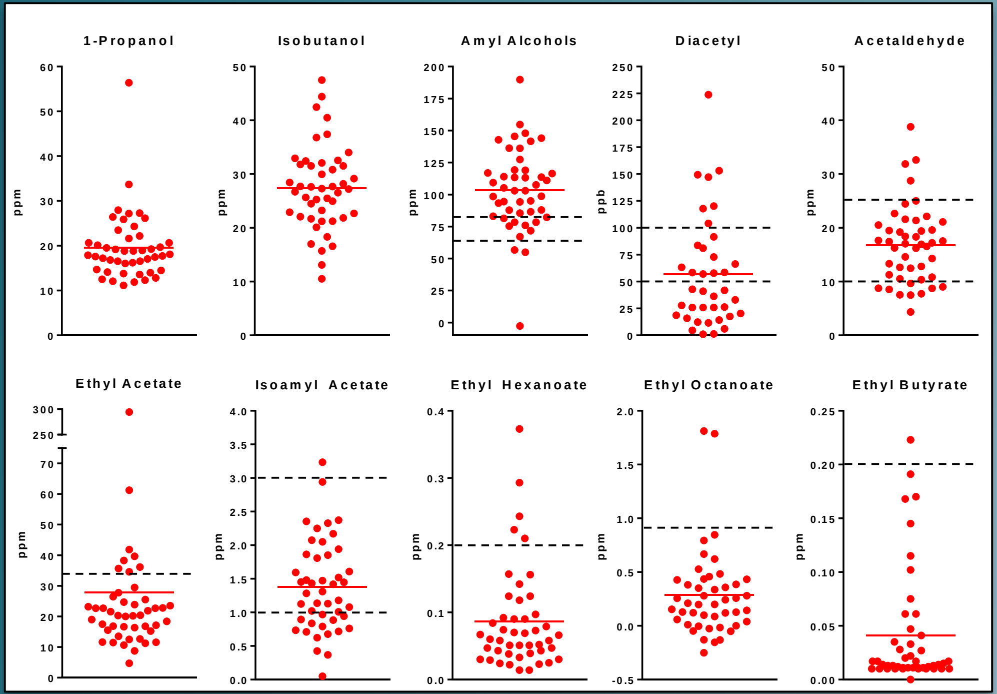
More complete experiments needed

2 weeks at 10 °C (50 °F)

Closed Fermentation

Open Fermentation

White Labs Analysis



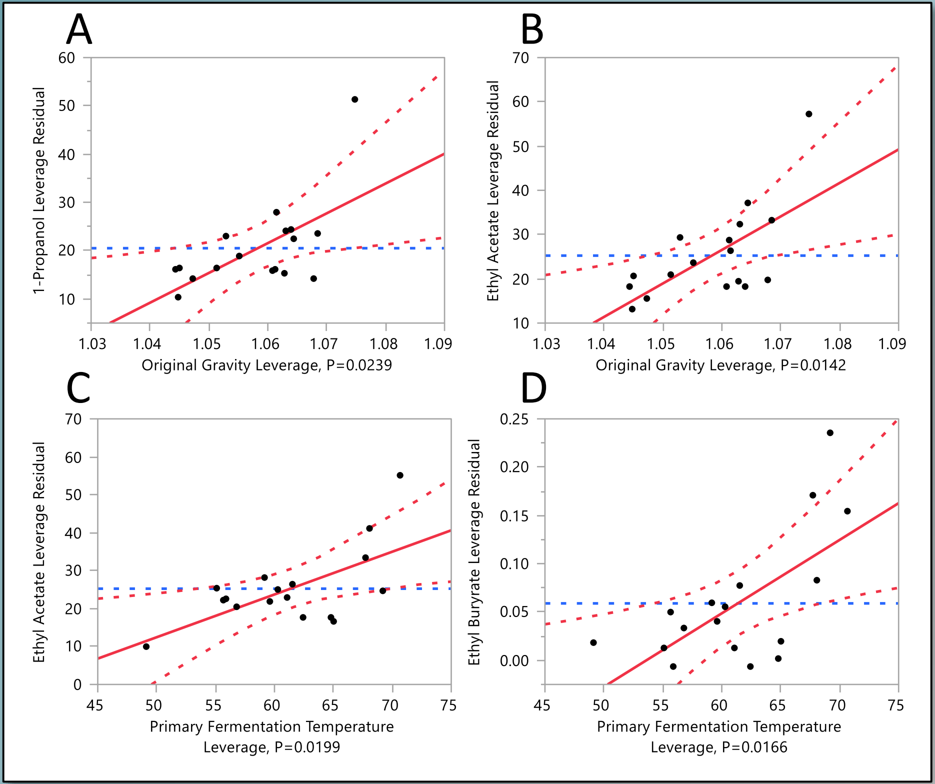
Compound	Detection Threshold	Flavor/Aroma
Diacetyl	50-100 ppb	butter or butterscotch
Acetaldehyde	10-25 ppm	green apples, raw apple skin, bruised apples
1-Propanol	700 ppm	fusel alcohol, solvent-like
Isobutanol	200 ppm	fusel alcohol, alcoholic, solvent-like
Amyl Alcohols	60-80 ppm	vinous, solvent-like
Ethyl Acetate	33 ppm	fruity with solvent undertones
Isoamyl Acetate	1-3 ppm	banana
Ethyl Hexanoate	0.2 ppm	apple like (ripe fresh), aniseed, pineapple, green banana
Ethyl Octanoate	0.9 ppm	apple, sweet, fruity, waxy, wine, floral, fruity, pineapple, apricot, banana, pear
Ethyl Butyrate	0.2-0.4 ppm	fruity, juicy fruit, pineapple, cognac, papaya

dashed lines indicate flavor threshold(s); 1-propanol and isobutanol levels all below threshold

Flavor Compounds DOE

Analyzed flavor compound outputs as function of OG, fermentation temperature, and fermentation time

Correlation between levels of 1-propanol and OG, ethyl acetate and OG, ethyl acetate and fermentation temp, ethyl butyrate and fermentation temp



What to Brew with *S. eubayanus*

The Patagonia Brewing Experience

Sensory information was gathered through a BJCP-sanctioned competition judged by Certified, National, and Grand Master judges

Mean score of 27 +/- 6 points and ranged from 13.5 to 39

Judging Observations/Comments

Grape or white wine aroma and flavor, apple and pear esters, and an artificial berry flavor at times

Phenols to varying degrees

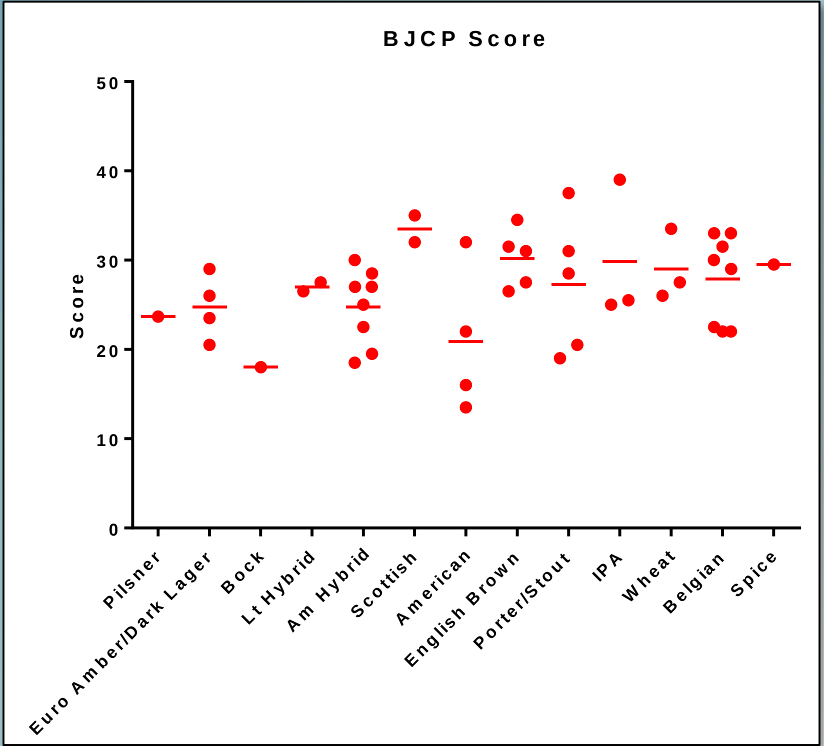
Lots of sulfur during fermentation, can stick around in beer

Biggest criticism was under-attenuation

"clean, crisp, inviting", "sock funk", "sour baby puke or rancid feet", "this beer/yeast combo was not a pleasant experience"

Works well with crystal and lightly roasted malts, pale beers, citrus American hops, Belgian-style

Beware of dark roasted malts, spicy hops



Winning Recipes

Eubayanus Brown Porter
Chris Clair

Batch Size (Gal): 5.50
Original Gravity: 1.048
Final Gravity: 1.020
Anticipated SRM: 28.4
Anticipated IBU: 29.2
Brewhouse Efficiency: 70 %
Wort Boil Time: 90 min
Saccharification Rest Temp: 152°F Time: 60 min
Sparge Temp: 170°F Time: 60 min
Malts
8.00 lbs. Maris Otter
1.00 lbs. Brown Malt
0.38 lbs. Chocolate Malt
0.38 lbs. Pale Chocolate Malt
0.25 lbs. Crystal 120L
0.25 lbs. Crystal 60L
Hops
1.00 oz. Glacier (5.70%) 60 min
1.00 oz. Glacier (5.7%) 10 min

Fermented 65 F 6 days, racked and 55 F for 7 days

Citra IPL/APA
Steve Groff

Batch Size (Gal): 7.50
Original Gravity: 1.055
Final Gravity: 1.018
Anticipated SRM: 4.77
Anticipated IBU: 52
Brewhouse Efficiency: 67 %
Wort Boil Time: 90 min
Protein Rest Temp: 135°F Time: 20 min
Saccharification Rest Temp : 148°F Time: 40 min
Sparge Temp : 170°F Time: 60 min
Malts
6.00 lbs. Pilsner Malt
10.00 lbs. Vienna Malt
3.75 oz. Acidulated Malt
Hops
1.75 oz. Citra (11%) 30 min
1.25 oz. Citra (11%) 10 min
1.25 oz. Citra (11%) flameout

Fermented for 15 days at 50°F (India Pale Lager) with a 2-day diacetyl rest at 65°F or 62°F (American Pale Ale)

Recommendations

Keep it simple

Mash low, but not too long
- 145-150°F
- 60-90 min

Keep the roasted malt restrained
- roasted barley clashes with the phenols
- chocolate and Carafa work well

Test different fermentation temperatures
- nuances in ester and phenol production
- Citra IPL (10 °C/50°F) and Citra APA (15.5°C/60°F) had 6-point difference

Add some sugar to dry it out

Choose your hops wisely
- Compensate by increasing the IBUs
- Try citrusy hops, be careful of spicy/herbal

Add another yeast
- *Brettanomyces* flavors pair well with *S. eubayanus* esters and phenols
- A clean *S. cerevisiae* strain can be used to dry out beer without affecting flavor

What to Brew with *S. eubayanus*

Split batch four ways

1. Wyeast 1056
   - Clean with some malt and subtle hops
2. *S. eubayanus*
   - 64% attenuation
   - Yeast dominates, slight malt and hops
3. \+ sucrose (0.005 points)
   - 68% attenuation
   - Cleaner than #2, less phenolic, more "refined"
   - Preference between 2 & 3 split
4. \+ *Brettanomyces bruxellenis* (WLP650)
   - 77% attenuation (still slowly fermenting)
   - Work well together, can taste qualities of both yeast

Eubayanus Pale Ale

97.5 % Pilsner Malt; 2.5% Crystal 120
- 64 °C (152 °F), 1.5 hour

Hops: 42 IBU, 0.79 BU:GU
Amarillo Hops
- 26 IBU @ 60 min, 9.4 IBU @ 10 min, 2.6 IBU @ 5 min

Azacca Hops
- 3.5 IBU @ 5 min

1 oz/11 gallons of Amarillo and Azacca steeped for 20 min at flameout

Fermented 7 °C (45 °F), 4 weeks, racked and lagered

SG: 1.052
FG: 1.022
58% attenuation
ABV: 4%

The Future of *S. eubayanus*

Commercial use of *S. eubayanus*

Great for homebrewing, but foresee problems at a commercial scale

Heineken licensed for 195k € + royalties

“When we started working with it, it just died on the spot…” Willem van Waesberghe, master brewer at Heineken

H41 released March 2016 in the Netherlands and Italy

“The new lager has a fuller taste, with spicy notes balanced by subtle fruity hints.”

https://www.youtube.com/watch?v=8hoV2JMgtL4

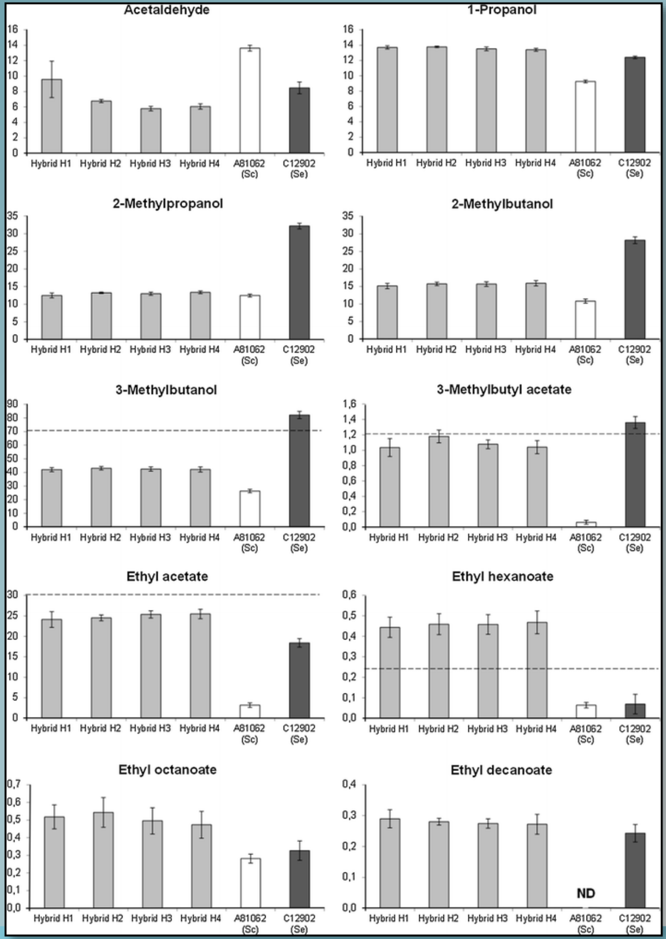
Yeast Breeding

Workaround for GM brewing yeast

Several groups created novel lager strains by crossing *S. cerevisiae* and *S. eubayanus*[1,2,3,4]

Select clones for whatever phenotype you want (attenuation, cold tolerance, fruitiness, phenolics, flocculation, etc)

Difficult getting rid of all phenolic flavor/aroma



1. Hebly et al. FEMS Yeast Res. May;15(3). 2015
2. Krogerus et al. J Ind Microbiol Biotechnol. May;42(5). 2015
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Thank You

Brett Baker & Krogerus Kristoffer

White Labs

BUZZ; Stoney Creek Homebrewers (Bryon Martinez); Keystone Hops (Andy Hejl)

Judges (Chris Clair, Dave Houseman, Dave Manning, Bryon Martinez, Bill McGeeney, Mark Prior)

Competition Sponsors - The Yeast Bay and Northern Brewer

www.shantybrewery.com for updates and other musings

Competition Participants

Earle Bare
Steve Bischoff
Tim Caum
Chris Clair
Chris Corbin
Ken Dickson
Kiel Fisher
Brendan FitzGerald
Chuck Golder
Steve Groff
Mike Hamara
Ken Harris
Andy Hejl
David Henderson
Dave Houseman
Tim Kepner
Mark Kissinger
Fred Kline
Jim Lachman
Rob Madden
Andy Maginnis
Frank Markley
Bryon Martinez
Bryan McClain
Brian Peck
Mark Prior
Bob Purrenhage
John Putnam
Matt Reeser
Alvaro Reyes
Cindy Serdikoff
Steve Shantz
Mike Smith
Mark Sofio
Mike Todd
Jeff Washeleski
Bob Weidenmoyer
Josh Weikert

NOTE: *S. eubayanus* is not commercially available, and may be obtained with a research license through the Portuguese Yeast Culture Collection (PYCC 6148).